DEMONSTRATION OF A SPIROCHETICIDAL EFFECT BY CHEMICAL CONTRACEPTIVES ON *TREPONEMA PALLIDUM*[1]

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Five contraceptive chemicals applied intravaginally and a vaginal germicide were tested for the ability to kill Treponema pallidum. *The results suggest that such products might play a useful role in preventing person-to-person transmission of syphilis.*

Introduction

The prevention of primary syphilis has always been of great concern to physicians, medical researchers, and public health workers. The general theme is one of considerable antiquity, but the first published material in medical literature was written by a Venetian physician named Fallopus in 1564, who suggested applying wine to the exposed area for prevention of syphilis. The first advocate of mercury was Agato, who suggested using it in 1733, and mercury preparations continued to command attention for centuries *(1)*. By 1904 Metchnikoff and Roux had discovered, through human and animal experiments, that calomel (mercurous chloride) was useful in preventing infection with *Treponema pallidum (2)*. Except for potassium iodide, mercury remained the only truly efficacious therapy known until Ehrlich introduced arsphenamine (salvarsan) into clinical practice in 1910 *(3)*.

The Venereal Disease Research Laboratory of the United States Public Health Service carried out research for many years to develop methods of chemical prophylaxis. Various metallic compounds were screened to determine their in vivo and in vitro spirocheticidal properties, and by 1950 a preparation containing orvus-mapharsen had been studied extensively and found suitable for chemical prophylaxis *(4)*. However, after World War II both civil and military programs decided to rely completely on therapy associated with well-developed case-finding methods. This policy caused a virtual cessation of research on venereal disease prophylaxis. (It is of interest to note that these studies on chemical prophylaxis were directed toward prevention of infection in males.)

The worldwide resurgence of gonorrhea and syphilis, despite the existence of specific and effective therapeutic agents, prompted our interest in investigating other possible methods, including topical prophylaxis, for the prevention of sexually transmissible diseases. There are certain indications suggesting that the modern oral contraceptives and intrauterine devices used by women, which do not provide any protection against disease, may be an important factor in the increased sexual transmission of diseases and the venereal disease epidemic *(5, 6, 7)*.

With these considerations in mind, a research project was begun to search for a vaginal preparation that would provide both contraception and prophylaxis against venereal disease *(8, 9)*. The present article, an extension of work published previously, describes experiments demonstrating that currently used vaginal chemical preparations have a spirocheticidal effect. These experiments involved both in vitro testing and infectivity tests in the rabbit, the standard test animal used for this purpose.

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Materials and Methods

The chemical contraceptives tested in these experiments were over-the-counter products that could be purchased directly from local stores in the United States. Dilutions (weight/volume) of various contraceptives were prepared in physiologic saline solution and a mechanical (vortex) mixer was used to ensure proper mixing.

Spirochete Harvest

The method used to harvest spirochetes, which has been reported previously *(9)*, was essentially as follows: For regular passage and maintenance of *T. pallidum*, 0.2 cc of fluid containing spirochetes was injected into a healthy normal rabbit by the intratesticular route. The spirochete harvest from this injected rabbit was then made in about 11 days, at the peak of acute orchitis.

At this time the rabbit was sacrificed by injecting air into the ear vein. The testicles were removed aseptically, sliced, and minced into small pieces. Physiologic saline solution containing 20 per cent rabbit serum was added in the amount of 15-20 cc for each pair of testicles.

Spirochetes were extracted by shaking the flask for one and a half hours, using a low-speed mechanical shaker at room temperature. The material was then centrifuged for 15 minutes at 110 x G, and the supernatant suspension of spirochetes was removed. The harvest was considered satisfactory for use in the tests if 10 or more actively motile spirochetes were present in each of five different 40x dark fields.

Testing for Spirocheticidal Effects

The spirocheticidal effects of five chemical contraceptives and a germicidal product were determined by the following methods:

1) In vitro method. Each product was diluted to 50, 20, 10, and 1 per cent concentrations (weight/volume) with physiologic saline solution. For purposes of preliminary screening, one drop of the *T. pallidum* suspension was placed on a microscope slide next to one drop of the diluted contraceptive or germicide sample. The two drops were mixed with an applicator stick and a stopwatch was started. A coverslip was then put in place and the slide was examined using a darkfield condenser. The time required to immobilize the spirochetes was noted and recorded.

Because one to one and a half minutes were required for the preparation of the slide and thorough darkfield examination of five different fields of each wet smear, only dilutions that were effective in rendering the spirochetes nonmotile within one to one and a half minutes were selected for confirmatory testing. This confirmatory testing used a more quantitative method in which 0.5 ml of *T. pallidum* suspension was mixed with an equal volume of an appropriate dilution of the contraceptive or germicide. Slides were then prepared and examined after varying time intervals; the motility of the spirochetes was noted; and the time required to immobilize the spirochetes at each particular dilution was recorded. Physiologic saline (0.9 per cent saline) solution was used as a control, and the number of spirochetes observed and their motility was recorded at the end of the experiment. The pH of the saline control solution was adjusted with 1N HCl or NaOH to match that of the test samples.

The number of spirochetes observed in each dark field was multiplied by a factor representing the magnification of the objective lens of the microscope, and an average of five fields (observation) was used to determine the "per cent motility."

2) Infectivity test in rabbits. Dilutions of the six products to be tested were prepared in physiologic saline. An aliquot of each dilution was then mixed with an equal volume of *T. pallidum* harvest (in 0.9 per cent saline solution with about 20 per cent rabbit serum) containing more than ten million motile spirochetes per ml. The infectivity of the mixture was determined by exposing the spirochete sample to each product for 10 minutes and inoculating two rabbits with each spirochete-product

mixture. In each case 0.2 ml of the mixture was injected into the right testicle; in addition, 0.2 ml of the product solution alone was injected into the left testicle for purposes of subsequent comparison. For positive control, two rabbits were injected intratesticularly with similar 0.1 ml and 0.2 ml samples of the spirochete harvest alone.

All the rabbits were then observed for signs of orchitis or other gross pathological change. Each rabbit was observed daily for 90 days, unless it developed orchitis confirmed by the presence of spirochetes on darkfield examination. If the inoculated rabbit failed to develop orchitis, and if microscopic examination of the testes showed no *T. pallidum,* the preparation was considered noninfectious.

Results

The results of in vitro testing of the six products and physiologic saline at various dilutions—in terms of the effect on *T. pallidum* motility after two and five minutes—are shown in Table 1. Of the five contraceptives, both Delfen cream and Ortho cream completely inhibited spirochete motility within two minutes of exposure at all dilutions. Koromex jelly was completely effective at 50 and 20 per cent dilutions. Ten per cent dilu-

Table 1. *T. pallidum* motility following two to five minutes' exposure to various concentrations of a contraceptive or Betadine Vaginal Gel. The figures shown represent the average percentages of motile spirochetes observed in five fields during darkfield examination.

Product tested	% concentration of product (wt./vol.) in physiologic saline	% motility of spirochetes after exposure for:	
		2 minutes	5 minutes
Delfen contraceptive cream (Ortho)	50	0	0
	20	0	0
	10	0	0
	1	0	0
Ortho-Creme contraceptive cream (Ortho)	50	0	0
	20	0	0
	10	0	0
	1	0	0
Koromex contraceptive jelly (Holland-Rantos)	50	0	0
	20	0	0
	10	50	20
	1	100	100
Emko vaginal foam contraceptive (Emko)	50	0	0
	20	0	0
	10	0	0
	1	30	20
Because birth control foam (Because Contraceptor, Emko)	50	0	0
	20	0	0
	10	0	0
	1	25	20
Betadine vaginal gel (Purdue Frederick)	50	0	0
	20	0	0
	10	0	0
	1	40	30
Physiologic saline (0.9% solution)		100	100

tions of this contraceptive rendered about half of the spirochetes inactive in two minutes and about four-fifths inactive after five minutes. One per cent dilutions of Koromex jelly did not appear to reduce the spirochete motility. Both Emko foam (aerosol foam in a large container under pressure) and Because foam (an unpressurized foam used with the Because Contraceptor inserter) were effective at 50, 20, and 10 per cent dilutions; however, 1 per cent dilutions did not immobilize all the spirochetes. After two and five minutes of exposure to 1 per cent dilutions of Emko foam and Because foam, about a fifth to a third of the spirochetes were still showing active motility.

Similarly, Betadine vaginal gel, a germicidal preparation, was effective at 50, 20, and 10 per cent dilutions, but not at 1 per cent. That is, about a third of the spirochetes remained motile after five minutes of exposure to a 1 per cent dilution of Betadine gel. The physiologic saline solution (0.9 per cent), used for control purposes, did not decrease the motility of the spirochetes even after five or more (up to 20) minutes of exposure.

Based on these in vitro observations, appropriate dilutions of each contraceptive and Betadine gel were selected to confirm the observed spirocheticidal activity of these products. The data in Table 2 show the response of rabbits to intratesticular inoculation with spirochetes exposed to an appropriate dilution of each substance.

The two rabbits inoculated with spirochetes unexposed to contraceptives or Betadine gel developed acute orchitis within 10 days. However, the 12 rabbits inoculated with the same spirochete harvest after the spirochetes had been exposed to one of the diluted products did not develop orchitis, even when observed for 90 days. A slight induration and nodular growth developed in the rabbit testicles inoculated with Betadine gel, but examination found this to be negative. Under darkfield examination, testicular biopsy material from the two rabbits with orchitis showed the presence of a large number of actively motile spirochetes, whereas no spirochetes were found in biopsy material from the rabbits without orchitis.

Discussion and Conclusions

Vaginal chemical contraceptives, also known as spermicides or topical contraceptives, provide the oldest known form of contraception. The active ingredients in these preparations are able to immobilize or destroy human sperm. In addition, many of these active ingredients also have antimicrobial properties. Some of the more recently developed contraceptives, such as foaming tablets (Penigin, Penigin C) now available in Japan, contain a combination of antibiotic and spermicidal agents.

The chemical group of detergents known as nonionic surfactants are another major class of compounds used as emulsifiers and often as active ingredients in formulating vaginal con-

Table 2. Infectivity of 0.2 ml samples of a *T. pallidum* harvest following 10 minutes' exposure to the tested contraceptive products, Betadine gel, and physiologic saline.

Type of contraceptive	% concentration of product (wt./vol.) in physiologic saline	No. of rabbits inoculated	No. of days observed	No. of rabbits developing orchitis
Delfen cream	1	2	90	0
Ortho cream	1	2	90	0
Koromex jelly	20	2	90	0
Emko foam	10	2	90	0
Because foam	10	2	90	0
Betadine gel	10	2	90	0
Physiologic saline		2	10	2

traceptives *(12)*. The mechanism of action of some bactericidal compounds is similar to that of surface-active agents, since some bactericides act by altering the bacteria's surface characteristics. Surfactants and bactericides together generally have a synergistic effect, making a combined product more effective as a spermicide than any one of the ingredients alone *(13)*.

Considering the properties of some of the active ingredients present in the spermicidal preparations tested in our experiments, it is not surprising to find that spirochetes were immobilized and even lysed (broken pieces of spirochetes were observed under higher magnifications with oil immersion and 100x objectives) when exposed to dilutions of these contraceptive products. In general, the degree of spirocheticidal activity reflected the quantity and quality of active ingredients present in the contraceptives. The strength of the diluted contraceptives that immobilized actively motile spirochetes within a short exposure time ranged from 1 to 20 per cent. With increasing length of exposure, fewer spirochetes survived. However, physiologic saline (with and without pH adjustment) had no evident effect on spirochete motility; that is, the spirochetes survived even after five minutes of exposure.

The infectivity test confirmed that samples of a *Treponema pallidum* harvest containing more than 10 million actively motile spirochetes per ml were noninfectious following exposure to diluted germicide and contraceptive preparations known to render them immobile. No orchitis was observed in rabbits receiving intratesticular inoculations with spirochetes treated with these diluted products, indicating that all the spirochetes were killed when the spirochete harvest was exposed. Small quantities of diluted contraceptives inoculated into rabbit testicles were well-tolerated, and no adverse signs or symptoms were observed in these animals. Some induration and nodular growth was observed in the testicles near the sites of inoculation with Betadine gel. This reflected a likely irritating effect of that preparation. However, no spirochetes were seen when material from these nodules was subjected to darkfield examination. In contrast, the rabbits infected with the spirochete harvest that was not exposed to a contraceptive or Betadine gel developed acute, generalized orchitis (with enlargement) within 10 days.

In general, infectivity testing is considered more sensitive than microscopic examination for detecting the presence of live and infectious *Treponema pallidum* spirochetes, since a single live *T. pallidum* injected into a rabbit via the intratesticular route under appropriate conditions will produce orchitis *(14)*.

Overall, these experiments confirm the spirocheticidal effect of five chemical contraceptives and suggest the potential usefulness of such intravaginal contraceptives in preventing person-to-person transmission of syphilis. However, the potential impact of chemical prophylaxis and the possible role of contraceptives in preventing syphilis can only be determined through well-designed clinical field studies.

SUMMARY

Five contraceptives marketed in the United States for intravaginal use, as well as a germicidal preparation (Betadine gel), were tested to determine their spirocheticidal effect on *Treponema pallidum*. Samples of *T. pallidum* harvested from infected rabbits—samples containing more than 10 million spirochetes per ml—were inactivated when exposed to the diluted contraceptives or germicide. The lowest contraceptive and germicide concentrations found effective in rendering spirochetes immobile ranged from 1 to 20 per cent.

These same concentrations rendered comparable spirochete samples noninfectious for rabbits inoculated by the intratesticular route. This infectivity testing thus confirmed the spirocheticidal properties of the products tested. Altogether, these results suggest that intravaginal contraceptives could play a useful role in the prevention of syphilis.

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